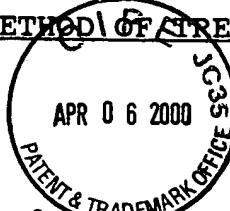


COMPOSITION AND METHOD OF TREATING HEPATITIS C



I. GOVERNMENT INTEREST

This invention described herein may be manufactured, used and licensed by or for the Government for governmental purposes without the payment to us of any royalties thereon.

II. RELATED APPLICATION

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/404,844 filed January 24, 1994, which is a continuation of U.S. Patent Application Serial No. 07/878,372 filed May 4, 1992 [which in turn is] a continuation in part of U.S. Patent Application Serial No. 07/759,544, filed September 13, 1991.

III. FIELD OF INVENTION

This invention relates generally to the pharmacological treatment of hepatitis C virus infection in patients.

IV. DESCRIPTION OF THE RELATED ART

Hepatitis C Virus (HCV), the putative agent in the majority of post-transfusion acquired hepatitis, has been recently defined by a new serologic assay. Kuo, G., et al., Science, 244:362-4 (1989). Despite improvement in the quality of the blood-donor pool and the recent implementation of testing of donated blood, the current estimated incidence of acute infection

1 among persons receiving transfusions is 5 to 10%.

2 Alter, H.J., in Zuckerman, A.J., ed., Viral Hepatitis
3 and Liver Disease, Allen K. Liss, New York, 1988,
4 pp.537-42. Chronic hepatitis develops in at least half
5 the patients with acute HCV infection (representing
6 about 90% of patients with non-A, non-B hepatitis
7 (NANB)), and cirrhosis develops in at least 20% of this
8 group. Thus, of the approximately 3 million persons
9 who receive transfusions in the United States each
10 year, acute hepatitis C will develop in about 150,000.
11 Chronic hepatitis C will develop in at least 75,000 of
12 these, and among them cirrhosis will develop in more
13 than 15,000. Among patients with post-transfusion
14 hepatitis, up to about 90% are positive for the HCV
15 antibody. Davis, G.L., et al., New England Journal of
16 Medicine, 321:1501-6 (1989). Patients with sporadic
17 NANB hepatitis (no specific risk factors) are also very
18 likely to have the anti-HCV antibody. Kuo, et al.
19 (1989) above. While most of the patients who contract
20 hepatitis C will have subclinical or mild disease,
21 approximately 50% will progress to a chronic disease
22 state characterized by fluctuating serum transaminase
23 abnormalities and inflammatory lesions on liver biopsy.
24 By some estimates, cirrhosis will develop in up to
25 about 20% of this group. Koretz, R.L., et al.,
26 Gastroenterology, 88:1251-4 (1985).

1 With the aim of halting or slowing the progression
2 of HCV-related diseases, a variety of drugs have been
3 evaluated in recent years. Both acyclovir and
4 corticosteroids (which are beneficial in autoimmune
5 chronic active hepatitis) are ineffective. Pappas,
6 S.C., J. Med. Virol., 15:1-9 (1985); Stokes, P., et
7 al., Gastroenterology, 92:1783 abstract (1987).

8 To date, α -interferon (IFA) appears to be the most
9 promising candidate, although not necessarily the final
10 answer. Hoofnagle, J.H., et al., in Viral Hepatitis:
11 1981 International Symposium, Philadelphia, Franklin
12 Institute Press, 1982, pp. 573-83; Hoofnagle, J.H., et
13 al., New England Journal of Medicine, 315:1575-8
14 (1986); Thomson, J., Lancet, 1:539-41 (1987); Kiyosawa,
15 K., et al., in Zuckerman, A., ed., Viral Hepatitis and
16 Liver Disease, Allen K. Liss, New York, 1983, pp. 895-
17 7. Hoofnagle, J.H., et al., Sem. Liver dis., 9:259-263
18 (1985). The interferons are host proteins made in
19 response to viral infections as well as other antigenic
20 stimuli. They are classified by their cell or origin
21 as well as their antigenicity. α -Interferon is made by
22 lymphoblastoid cells, β -interferon by fibroblasts, and
23 γ -interferon by T-cells. Subtypes in each group are
24 based on antigenic/structural characteristics.
25 Recombinant forms for each group have been developed
26 and are commercially available. A pilot study

1 utilizing IFA on ten patients with well-characterized
2 post-transfusion NANB hepatitis was reported in 1986 by
3 Hoofnagle et al. (Hoofnagle, J.H., et al., New England
4 Journal of Medicine, 315:1575-8 (1986)). In this
5 study, eight of ten patients improved their serum
6 alanine transaminase (ALT) levels within one month of
7 starting therapy. IFA therapy consisted of 5 million
8 units (MU) daily in seven of the patients and one MU
9 daily in three patients. In all subjects the dose was
10 gradually reduced to 1 MU daily and then finally
11 switched to an alternate day or every three day
12 regimen. In three patients who had post-treatment
13 liver biopsies, the specimen showed a marked
14 improvement in the degree of portal inflammation and
15 loss of parenchymal hepatocytic necrosis. Side effects
16 were common at the 5 MU/day dose and virtually absent
17 at 1 MU/day.

18 The effects of recombinant human interferon α in a
19 prospective, randomized, double-blind, placebo-
20 controlled trial in patients with well-documented
21 chronic HCV infection has recently been carried out.
22 Di Bisceglie, A.M., et al., New England Journal of
23 Medicine, 321:1506-10 (1989). Forty-one patients were
24 enrolled in the trial, 37 of whom were later found to
25 have antibody to HCV. Twenty-one patients received
26 interferon α (2 MU) subcutaneously three times weekly

1 for six months, and twenty received placebo. The mean
2 serum ALT and the histological reatures of the liver
3 improved significantly in the patients treated with
4 interferon, but not in the patients given placebo. Ten
5 patients treated with interferon (48%) has a complete
6 response, defined as a decline in mean serum ALT to the
7 normal range during therapy; three others had a
8 decrease in mean ALT of more than 50%. After treatment
9 ended, however, serum ALT usually returned to
10 pretreatment levels; six to twelve months after the
11 discontinuation of interferon therapy, only two
12 patients (10%) still had normal values. The authors
13 concluded that interferon α therapy is beneficial in
14 reducing disease activity in chronic hepatitis C;
15 however, the beneficial responses are often transient
16 and side effects are known to appear.

17 In another, broader study, chronic hepatitis C
18 (NANB hepatitis) is 166 patients was treated with
19 either 3 MU or 1 MU of recombinant human α -IFA three
20 times weekly for 24 weeks or to no treatment. The
21 serum ALT level became completely normal in 22 of the
22 26 patients (85%) who responded to treatment with 3 MU
23 of interferon, and nine of the sixteen patients (56%)
24 responded to treatment with 1 MU. The patients who
25 received 3 MU of interferon had histologic improvement
26 because of the regression of lobular and periportal

1 inflammation. However, relapse within six months after
2 the completion of treatment occurred in 51% of the
3 patients treated with 3 MU of interferon and in 44% of
4 those treated with 1 MU. Davis, G.L., et al., New
5 England Journal of Medicine, 321:1501-06 (1989). These
6 authors concluded that a 24-week course of interferon
7 therapy is effective in controlling disease activity
8 in many patients with hepatitis C, although relapse
9 after the cessation of treatment is common.

10 A multi-center randomized control trial of
11 recombinant human α -IFN in patients with chronic NANB
12 hepatitis has been reported recently. Marcellin, P.,
13 et al., Hepatology, 13:393-97 (1991). Patients were
14 randomly assigned to no treatment or to 1 to 3 MU of α -
15 interferon given three times a week for 24 weeks.
16 Forty-five patients (75%) were positive for antibody to
17 HCV. During the 24 week treatment period, mean serum
18 ALT levels decreased in both treatment groups, but the
19 decrease was statistically significant only in the 3 MU
20 group. However, at 24 weeks, the proportion of
21 patients with normal ALT levels was similar in the 3 MU
22 group (39%) and the 1 MU group (45%) and both were
23 significantly higher than in controls (0%). Repeat
24 liver biopsy specimens showed a significant decrease in
25 the severity of histological changes in the higher dose
26 group but not in the lower dose group or in controls.

1 However, after treatment, the mean ALT levels rose in
2 both treated groups. The proportion of patients with
3 normal ALT levels at week 48 was 28% in the 3 MU group
4 and 20% in the 1 MU group. The authors conclude that a
5 dose of 3 MU was superior to 1 MU of α -interferon given
6 three times per week for 24 weeks in inducing
7 improvements in serum ALT levels and liver histological
8 examinations. However, relapse in disease activity
9 occurred in approximately half of the responders when
10 interferon was stopped. The response to α -interferon
11 did not correlate with the source of infection or
12 with the presence or absence of anti-HCV antibody titres
13 in patient sera.

14 It is clear, therefore, that while α -interferon
15 has a beneficial effect on the course of HCV infection,
16 this effect is frequently only transient. therefore,
17 new modalities are necessary in order permanently to
18 eradicate the effects of hepatitis C virus on the
19 patient.

20 Another class of polypeptide immune modifiers
21 derived from the thymus gland, the thymosins, has been
22 shown to trigger maturational events in lymphocytes, to
23 augment T-cell function and to promote reconstitution
24 of immune defects. Low, T.L.K., et al., "Thymosins:
25 Structure, Function and Therapeutic Application",
26 Thymus, 6:27-42 (1984).

1 Thymosin Fraction Five (TF-5), originally
2 described by Goldstein et al. (Proc. Nat'l Acad. Sci.
3 (USA), 69:1800-1803 (1972)), is a partially purified
4 extract of bovine thymus containing at least 40 peptide
5 components, 20 of which have been purified to
6 homogeneity or near homogeneity; it contains about 0.6%
7 of Thymosin α -1 (THN α ₁). Low, 1984, above.

8 THN α ₁, initially isolated from TF-5, has been
9 sequenced and chemically synthesized. Wetzel, R., et
10 al., Biochemistry, 19:6096-6104 (1980). Its sequence
11 is highly homologous in mice, calves and humans. THN α ₁
12 is a 28 amino acidic polypeptide with a molecular
13 weight of 3100 that has shown activity qualitatively
14 similar to TF-5 in modulating the immune system. Low,
15 T.L.K., et al., J. Biol. Chem., 254:981-6 (1979).
16 THN α ₁ has potent immunologic activity, including
17 stimulation of α - and γ -interferon production,
18 increasing macrophage migration inhibitory factor
19 production, inducing expression of T-cell markers,
20 including IL-2 receptors, and improving T-cell helper
21 cell activity. Schulor, R.S., et al., in The
22 Lymphocyte, Allen J. Liss Inc., New York, 1981, pp.
23 191-215; Low, T.L.K., et al., in "Thymosins: Structure,
24 Function and Therapeutic Applications", Thymus, 6:27-43
25 (1984); Koutab, N.M., et al., Immunopharm., 16:97-105
26 (1988). Studies in mice have demonstrated a

1 synergistic effect of $\text{THN}\alpha_1$ and interferon on natural
2 killer-cell activity in immunosuppressed mice.
3 Favilli, C., et al., Cancer Immunol. Immunother.,
4 20:189-92 (1985). TF-5 and $\text{THN}\alpha_1$ can influence
5 immunoregulatory T-cell function, promote production of
6 interferon- α , interferon- γ and interleukin-2 by human
7 lymphocytes and increase interleukin-2 receptor
8 expression. Marshall, G. D., et al., J. Immunol.,
9 126:741-4 (1981); Mutchnick, M.G., et al., Clin.
10 Immunol. Immunopathol., 23:626-33 (1982); Sztein, M.B.,
11 et al., Proc. Nat's Acad. Sci. (USA), 83:6107-6111
12 (9186); Serrate, S.A., et al., J. Immunol., 1939:2338-
13 43 (1987); Bazevanis, C.N., et al., Immunopharm.,
14 13:133-41 (9187); and, Svedersky, L.P., Eur. J.
15 Immunol., 12:244-7 (1982).

16 Clinical trials of TF-5 and $\text{THN}\alpha_1$ as primary or
17 adjunctive therapy in patients with immunodeficiency or
18 cancer indicate that these agents enhance immune
19 responsiveness and augment specific lymphocyte
20 functions. Clinical trials of TF-5 and purified $\text{THN}\alpha_1$
21 have been underway for a number of years. Early trials
22 in patients with cancer or immunodeficiency states were
23 encouraging, though not definitive. Goldstein, A.L.,
24 et al., Transp. Proc., 9:1141 (1977); Barrett, D.J., et
25 al., J. Pediatr., 97:61 (1980); and Cohen, M.H., et
26 al., J. Amer. Med. Assoc., 241:1813-5 (1979). $\text{THN}\alpha_1$

1 use has been described in a randomized trial of
2 patients with nonsmall cell lung cancer. Patients were
3 treated with THN α_1 at a dose of 900 μ grams/m²
4 subcutaneously twice weekly or daily for two weeks and
5 then twice weekly after completing a course of
6 radiotherapy. The only side effect of THN α_1 was mild
7 burning at the injection site in three patients. This
8 was attributed to the drug lot and may have been due to
9 the carrier preparation. Relapse-free survival and
10 overall survival were greater in both THN α_1 treatment
11 groups than in the placebo group; some restoration of
12 radiation-suppressed immune function was also noticed.
13 There was no increase in T-cell numbers associated with
14 this. Schulof, R.S., et al., J. Biol. Response
15 Modifiers, 4:147-58 (1985).

16 Recent double-blind, randomized trials with
17 thymosins have been performed in elderly men in an
18 effort to increase response to influenza vaccine.
19 Gravenstein, S., et al., JAGS, 37:1-8 (1989). Patients
20 received synthetic THN α_1 subcutaneously twice weekly
21 starting at the time the influenza vaccine was given.
22 At six weeks post-vaccine, those patients randomized to
23 receive the drug had higher levels of antibody to
24 influenza than controls. This difference was
25 accentuated in the very elderly (ages 77-99). No

1 clinical or biochemical toxicity was observed in drug
2 recipients.

3 There are preliminary reports that thymosins may
4 be effective against infections caused by hepatitis
5 viruses other than HCV. In an animal model of viral
6 hepatitis, the woodchuck infected with the Woodchuck
7 Hepatitis Virus, THN_α, suppressed viral DNA
8 replication, but produced no improvement in clinical
9 parameters. Korba, B.E., et al., Hepatology, 12:Abs.
10 880 (1990). In a pilot clinical trial with patients
11 with Chronic Active Hepatitis B caused by the hepatitis
12 B virus (HBV), patients treated for a year with THN_α,
13 (5 patients) or with TF-5 (2 patients) showed a marked
14 decrease in serum ALT; 6 of the 7 patients also showed
15 reduced levels of serum HBV DNA, and 5 of 6 patients
16 initially positive for serum hepatitis B surface
17 antigen (HBsAg) subsequently cleared this antigen.
18 Mutchnick, M.C., et al., Hepatology, 10:Abs. 575
19 (1989). No suggestion was made in these abstracts that
20 the thymosins would be effective against any other
21 hepatitis viruses.

22 There remains, therefore, an important need in the
23 art for a new modality for the treatment of HCV
24 infections in mammals; this modality is disclosed
25 below.

26 SUMMARY OF THE INVENTION

1 A treatment modality for HCV infections has been
2 devised comprising the administration to mammals of
3 immune system-potentiating doses of one or more
4 thymosins in combination with interferon therapy.

5 It is thus an object of this specification to
6 disclose compositions and methods for the treatment of
7 acute or chronic HCV infections in mammals comprising
8 combination therapy with one or more thymosins and one
9 or more interferons.

10 This and other objects will become apparent by
11 reference to the specification and to the appended
12 claims.

DESCRIPTION OF THE INVENTION

14 A novel modality for treating HCV infection in
15 mammals has been devised, comprising the administration
16 to such mammals of one or more thymosins at doses which
17 potentiate immune responses, in combination with anti-
18 viral doses of one or more interferons.

19 By the term "thymosins" is meant any or all of the
20 immune system potentiating polypeptides naturally
21 occurring in the thymus gland or produced by chemical
22 or recombinant means, or fragments derived from any of
23 these polypeptides. By the term "mammals" is meant any
24 mammalian subject, including human and animal patients,
25 requiring treatment for hepatitis C infection.
26 "Mammal" and "subject" are used interchangeably.

1 Thymosin preparations suitable for treating HCV
2 infections include TF-5, THN α , and fragments thereof,
3 e.g., C-terminal 4-28 and 15-28, and N-terminal 1-8, 1-
4 14 and 1-20 fragments. These may be obtained from
5 Alpha-1 Biomedicals Inc., Foster City, California.

6 Subjects, e.g., human patients, may receive the
7 thymosin by subcutaneous injection or infusion, at
8 appropriate intervals for an appropriate period of
9 time. The thymosin is administered to mammals infected
10 with hepatitis C virus in amounts which facilitate or
11 promote in vivo inactivation of hepatitis C virus. A
12 pharmaceutical dosage unit of an immune system-
13 potentiating amount of a thymosin, such as TF-5, can be
14 from about 900 to about 1200 mg/m² body surface area in
15 a pharmaceutically acceptable carrier. A
16 pharmaceutical dosage unit of an immune system-
17 potentiating amount of a thymosin, such as THN α , or
18 immune system-potentiating fragments thereof, can be
19 from about 900 to about 1200 μ g/m² body surface area in
20 a pharmaceutically-acceptable carrier. Lyophilized
21 preparations of thymosins or fragments which contain
22 mannitol and phosphate buffer are dissolved in diluent
23 period to dispensing. Thymosins in diluent should
24 remain stable for at least six months when stored in a
25 refrigerator. It is convenient to dispense thymosin
26 solutions in one ml dose vials per month.

1 For a typical human patient, an administration
2 regimen of twice weekly (e.g., Monday and Thursday)
3 subcutaneous injection of about 1500 to about 1700 μ g
4 of THNa, or fragments therefrom is convenient. Dosages
5 and length of treatment can be flexible, and can be
6 determined by the subject's clinical response to the
7 thymosins.

8 The course of the disease and its response to drug
9 treatments may be followed by clinical examination and
10 laboratory findings. As elevated serum alanine
11 aminotransferase (ALT) and aspartate aminotransferase
12 (AST) are known to occur in uncontrolled hepatitis C,
13 and as a complete response to treatment is generally
14 defined as the normalization of these serum enzymes,
15 particularly ALT (Davis, G.L., et al., New England
16 Journal of Medicine, 321:1501-6 (1989)), progress of
17 treatment with thymosins is conveniently followed by
18 this art-recognized test performed, e.g., on a
19 sequential multiple analyzer.

20 Another means of evaluating subjects having
21 antibodies to HCV (not all subjects with hepatitis C
22 have detectable antibody to HCV - Weiner, A.J., et al.,
23 Lancet, 335:1-3 (1990)) is to periodically test
24 subjects' sera for the titer of these antibodies.
25 Anti-HCV antibodies may be tested by the currently
26 available C 100-3 test (Kuo, G., et al., Science,

1 244:362-4 (1989)), by an Elisa test (Ortho Diagnostic
2 Systems, Raritan, N.J.) or by a recombinant assay
3 (RIBA-1 and RIBA-2, Chiron Corporation, Emeryville,
4 CA). Any suitable test may be used.

5 In order to follow the course of HCV replication
6 in subjects in response to drug treatment, HCV RNA may
7 be measured in serum samples by, for example, a nested
8 polymerase chain reaction assay that uses two sets of
9 primers derived from the NS3 and NS4 non-structural
10 gene regions of the HCV genome. Farci, P., et al., New
11 England Journal of Medicine, 325:98-104 (1991); Ulrich,
12 P.P., et al., J. Clin. Invest., 86:1609-14 (1990).

13 Other appropriate laboratory tests to follow the
14 course of treatment are listed in Example 1 below.

15 Thymosin therapy is preferably used in combination
16 with interferon therapy, thereby combining the immune
17 system potentiating effect of thymosins with the anti-
18 viral effects of the interferons. An improved response
19 rate at the currently used interferon doses would be
20 beneficial, particularly in the light of dose-limiting
21 side effects at higher doses of these proteins. An
22 offshoot of this concept is the ability to achieve
23 comparable efficacy with interferon plus thymosin at
24 lower doses than would be required with interferon
25 alone.

1 In this combination therapy regimen, one or more
2 interferons (for example, recombinant interferon α -2b,
3 Intron-A, Schering-Plough, Kenilworth, New Jersey) is
4 (are) administered subcutaneously to subjects, e.g.,
5 human patients, at doses ranging between about 1 MU and
6 3 MU along with or sequentially with one or more
7 thymosins, preferably including THN α_1 , at a dose of
8 about 900 to about 1200 $\mu\text{g}/\text{m}^2$ body surface area.

9 Although the example above speaks in terms of
10 recombinant interferon α -2b, other anti-HCV-effective
11 interferons such as α -, β - and γ -interferons,
12 recombinant or naturally occurring, may be
13 advantageously used in this invention.

14 This combination dose regimen is flexible, and
15 depends on the clinical condition of the subject.
16 Where subjects are refractory to the preferred dosage
17 levels, these may be increased within the limits
18 dictated by undesirable side effects. Typically,
19 injections are made five times per week and continue
20 until an acceptable response by the subject is
21 realized.

22 Tests to determine the effectiveness of the
23 combination therapy may be the same as those described
24 above for thymosin treatment alone. In addition,
25 histological examination of liver biopsy samples may be
26 used as a second major criteria for evaluation.

1 Knodell, R.G., et al., Hepatology, 1:431-5 (1981),
2 whose Histological Activity Index (portal inflammation,
3 piecemeal or bridging necrosis, lobular injury and
4 fibrosis) provides a scoring method for disease
5 activity.

6 The following examples are provided merely to
7 illustrate the invention, and are not to be construed
8 in any way as limiting the scope of invention as set
9 forth in the specification and claims.

10 EXAMPLE 1

11 Preparation of Injectable Formulation

12 Pharmaceutical dosage units or 1 ml each are
13 prepared from the ingredients shown in Table 1 below.

14 TABLE 1

	<u>Active Ingredient</u>	<u>Amount Per mL</u>
16	Thymosin α -1	0.0016 g
<u>Inactive Ingredients</u>		
18	mannitol, U.S.P.	0.050 g
19	sodium phosphate dibasic, 20 heptahydrate, U.S.P.	0.002 g
21	sodium phosphate monobasic, 22 monohydrate, U.S.P.	0.0005 g
23	sodium phosphate dibasic, 24 2 mg/ml solution	
25	sodium phosphate monobasic, 26 0.5 mg/ml solution	
27	water for injection, U.S.P.	
28		

1

EXAMPLE 2

2

3

Treatment of Hepatitis C Infections in
Human Patients with Thymosins and Interferons

4

Adult patients with chronic active hepatitis C (CAHC) are randomized to one of four study groups, made up of about 40 patients per group. Selection criteria include: (1) patients are adults (at least 18 years of age); (2) serum ALT is elevated for at least six months prior to treatment with at least one value greater than twice the upper limit of normal in the laboratory doing the testing; (3) patients test positive for HCV antibody on two occasions and on a confirmatory test; and (4) liver biopsy within three months of treatment exhibits pathology consistent with chronic active hepatitis.

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Exclusion criteria include: (1) recent use of other anti-viral or immunosuppressive medication; (2) hemophilia, pregnancy or HIV infection, or other serious illness that could prevent completion of the course of treatment; (3) other forms of liver disease, including hepatitis A or B, α -1 antitrypsin deficiency, Wilson's disease, and hemochromatosis must be absent; (4) autoimmune markers (ANA, ASMA, AMA, anti-LKM1) must be absent or, if present, titers should be < 1:40; (5) leukocyte deficiency (<3,000); (6) low absolute neutrophil count (<1,000); (7) low platelets (<75,000);

1 (8) low Hb (<11 g/dL); (9) high bilirubin (>4 mg/dL);
2 and (10) low serum albumin (3 g/dL).

3 The first of the four randomized groups receives
4 interferon, preferably interferon α -2b, at a dose of 3
5 million units (MU) subcutaneously (SQ) on Mondays,
6 Wednesdays and Fridays, and receives placebos on
7 Tuesdays and Saturdays. The second group receives the
8 same dose/schedule of interferon, plus a thymosin,
9 preferably THNa₁, at a dose of 900 μ g/m² SQ on Tuesdays
10 and Saturdays. The third group receives the same
11 dose/schedule of a thymosin alone. The fourth group
12 receives placebo treatment initially, but can be
13 randomized to the three treatment groups thereafter.
14 Interferons and thymosins can be recombinant.

15 Patients begin treatment while hospitalized for
16 about one week, during which period side-effects are
17 monitored.

18 Outpatient follow-up is initially at one week
19 intervals for two weeks, then at two week intervals for
20 two months, and then monthly for the remainder of the
21 treatment period. At each visit the following lab
22 tests are performed: CBC, platelet count, differential
23 and ESR, ALT, AST, GGT, alkaline phosphatase,
24 bilirubin, total bilirubin/albumin and HCV antibody.
25 At monthly intervals serum γ -globulin, TSH, ANA and
26 ASMA are assessed.

1 Drug toxicity is monitored on an ongoing basis
2 using both clinical and laboratory parameters.

3 Within one month of completing the initial six
4 months of treatment, patients undergo liver biopsy for
5 pathological examination according to Knodell et al.
6 above. This system provides a numerical scoring system
7 of histological activity in patients with asymptomatic
8 CAH.

9 At this time, control patients are randomized into
10 three groups to receive one of the three treatment
11 modalities, assuming that they still have CAH on
12 follow-up liver biopsy, and that one arm of the study
13 does not show highly significant positive or negative
14 results on analysis at six months.

15 Patients in the treatment groups are followed to
16 evaluate recrudescence of disease as evidenced by
17 rising ALT levels. Patients who showed response in the
18 initial six month treatment period, but who have a
19 recurrence of the disease thereafter, are provided with
20 additional therapy.

21 Additional serum or tissue tests are performed if
22 possible: evaluation of antibodies to interferons and
23 thymosins, polymerase chain reaction amplification of
24 hepatitis C genome segments in liver biopsy samples,
25 and quantitative evaluation of anti-hepatitis C serum
26 titers.

EXAMPLE 3

1 The treatment protocol is as in Example 2, except
2 that the interferon is used at the level of 2 MU, and
3 the thymosin at 1050 $\mu\text{g}/\text{m}^2$.

EXAMPLE 4

5 The treatment protocol is as in Example 3, except
6 that 1 MU of the interferon and 1200 $\mu\text{g}/\text{m}^2$ of the
7 thymosin are used.

EXAMPLE 5

10 Analysis of Data

11 There are two primary criteria for response to
12 therapy- normalization of ALT levels by the end of the
13 treatment period (a partial response may be defined as
14 a decrease of at least 50% of initial ALT), and
15 histological improvement as determined by the
16 Histological Activity Index (HAI) of Knodell et al.
17 above.

18 This analysis provides a raw score ranging from 1
19 to 22 per sample. Paired data can be analyzed using
20 the Wilcoxon paired-sample test. Additionally, samples
21 can be classified into mild, moderate or reverse CAH,
22 and improvement assessed using the Chi-square
23 statistical analysis.

24 Life-table analysis is used to evaluate remission
25 and relapse status in terms of normalization of ALT
26 levels. Other continuous variables are analyzed using